

Status of *Logania imbricata* (Guillaumin) Steenis & Leenh. (Loganiaceae)

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Abstract

Molecular systematics has clarified the taxonomic status of *Logania imbricata* (Guillaumin) Steenis & Leenh. as being a species of the genus *Geniostoma* J.R.Forst. & G.Forst., with the new combination ***Geniostoma imbricatum*** (Guillaumin) C.S.P.Foster & B.J.Conn made here.

Introduction

Guillaumin (1953) incorrectly assigned his New Caledonian species *Nautophylla imbricata* Guillaumin to the family Epacridaceae. Leenhouts and van Steenis (1962) correctly transferred this taxon to the Loganiaceae. These authors concluded that this taxon was best placed within *Logania* because of the papillose internodes and shortly ciliate calyx lobes, all morphological features commonly found within the latter genus. However, these features also occur in *Geniostoma*.

A recent systematic treatment of the intergeneric relationships within the tribe Loganieae (Loganiaceae) by Gibbons *et al.* (2012) was unable to support the monophyly of *Logania*, even though both *L.* section *Logania* and *L.* section *Stomandra* were resolved as monophyletic. As part of a study to test the monophyly of *Logania* and to develop a species phylogeny, an increased number of *Logania* taxa and additional molecular markers are being investigated. With the exception of two species, *Logania* is currently considered to be confined to Australia (Conn 1994; 1995). The status of the New Zealand species, *L. depressa* Hook.f., is uncertain, with the type and protologue inadequate for detailed assessment. This taxon is regarded as extinct (Anonymous 2003+). The status of the other non-Australian species, *L. imbricata* (Guillaumin) Steenis & Leenh. from New Caledonia, had already been queried by Conn (1995) and its phylogenetic placement is reevaluated here.

Materials, Methods and Data Analysis

Nucleotide sequences for the chloroplast *petD* gene region of *Geniostoma*, *Logania*, *Mitrasacme*, *Mitreola*, *Phyllangium*, *Schizacme* and several outgroup taxa (*Neuburgia*, *Spigelia*, and *Strychnos*) were downloaded from GenBank (see Gibbons *et al.* 2012). A specimen of *Logania imbricata* (*G. McPherson 19383 et al.*; NSW905713, full details below) was used for DNA extraction (GenBank Accession Identifier: KC537758).

To determine the placement of *Logania imbricata* within the Loganieae phylogeny, genomic DNA was extracted and the *petD* gene region was amplified using the polymerase chain reaction (PCR). PCR was carried out using the P_{ipetB1365F} and P_{ipetD738R} primers and thermal cycling conditions of Lohne and Borsch (2005), which amplify the *petB-petD* intergenic spacer, *petD* 5' exon (coding for the cytochrome b6/f complex subunit IV) and *petD* group II intron. Reactions were carried out in a total volume of 25 μ L using 12.5 μ L of EconoTaq PLUS GREEN 2X Master Mix (Lucigen), 1.25 μ L of each primer (final concentration of 1 μ M in solution), 2–5 μ L of template genomic DNA, and 5–8 μ L of molecular grade water. PCR products were visualised using 1.5% agarose gel electrophoresis and cleaned using Exonuclease I and Antarctic phosphatase according to the manufacturer's protocol, before being sequenced under BigDye terminator cycling conditions by Macrogen (Seoul, South Korea).

The forward and reverse sequences for the *petD* region of *L. imbricata* were assembled into a contiguous sequence using SEQUENCHER version 4.5 (Gene Codes Corporation, Ann Arbor, MI) and aligned with the sequences from GenBank using MUSCLE (Edgar 2004), followed by manual adjustments when the automatic alignments resulted in minor inconsistencies. To allow subsequent phylogenetic analysis, the appropriate nucleotide substitution model for the data was determined in ModelGenerator version 0.85 (Keane *et al.* 2006) using the Bayesian information criterion (BIC). The model that best fitted the *petD* region was shown to be the transversion model with gamma-distributed rate variation among sites (TVM+ Γ), the next best being the general time-reversal model with gamma-distributed rate variation among sites (GTR+ Γ). The data were analysed using maximum likelihood in MEGA version 5 (Tamura *et al.* 2011). As the TVM+ Γ model cannot be implemented in MEGA, the GTR+ Γ model was used. Support for clades was provided by non-parametric bootstrap (BS) analysis (Felsenstein 1985) with 1000 pseudoreplicates. For the purposes of discussion, nodes with BS values of 85–90 were defined as moderately supported and those ≥ 90 as strongly supported.

Bayesian phylogenetic analyses were carried out in BEAST version 1.7.4 (Drummond and Rambaut 2007) with support values provided by estimation of posterior probabilities. Posterior probabilities (PP) ≥ 0.95 were considered to provide support. The analysis was carried out with the TVM+ Γ model implemented and run for 10,000,000 generations, with samples taken every 1000 generations. This was confirmed in Tracer version 1.5 (Rambaut and Drummond 2009) to be an adequate number of generations, with effective sample size values for all parameters well over the recommended minimum of 200. The first 10% of trees (1000 trees) was discarded as burn-in.

Results and Discussion

Logania imbricata is placed within a strongly supported clade (PP=1.0; BS=96%) comprising members of the genus *Geniostoma* (Fig. 1). Research into resolving the status of the subgeneric classification of *Logania* is ongoing, but these results unequivocally demonstrate that *L. imbricata* belongs in *Geniostoma*.

The inclusion of *L. imbricata* in *Geniostoma* is also supported by the following morphological features: (1) corolla yellow-green (in New Caledonia, the corolla of *Geniostoma* is often greenish; *Logania* – white or yellow); (2) corolla lobes \pm erect, frequent in species of *Geniostoma* from New Caledonia (*Logania* – tending to be usually more spreading, hence corolla often \pm campanulate); (3) capsules septifragal in *Geniostoma* (*Logania* – septicidal); (4) seeds appear to be embedded in fleshy placenta, as in *Geniostoma* (*Logania* – fleshy placenta absent) (for detailed circumscription of these two genera see Conn 1980; 1994; 1995).

Nomenclature

Geniostoma imbricatum (Guillaumin) C.S.P.Foster & B.J.Conn, *comb. nov.*

Basionym: *Nautophylla imbricata* Guillaumin (1953) *Mémoires du Muséum National d'Histoire Naturelle, Paris, Série B, Botanique* 4: 44, fig. 13.

Synonym: *Logania imbricata* (Guillaumin) Steenis & Leenh. (1962) *Bulletin du Jardin Botanique de l'État à Bruxelles* 32: 440.

Type: New Caledonia: Province Sud: Arête meridionale du Mt Humboldt: *Virot* 432, décembre 1940 (holo.: P; iso: P).

Description: for a detailed description, see Guillaumin (1953).

Selected specimens examined: **New Caledonia:** Province Sud: Mt Humboldt, *H.S. McKee* 5417, 10 Oct 1956 (K, NSW823136); Contrefort Sud du Humboldt, *H.S. MacKee* 27914, 8 Dec 1973 (NSW823135, P); Mont Kouakoué, *H.S. MacKee* 32437, 9 Dec 1976 (NSW840989, P); Slopes of Mont Humboldt, *G.D. McPherson*

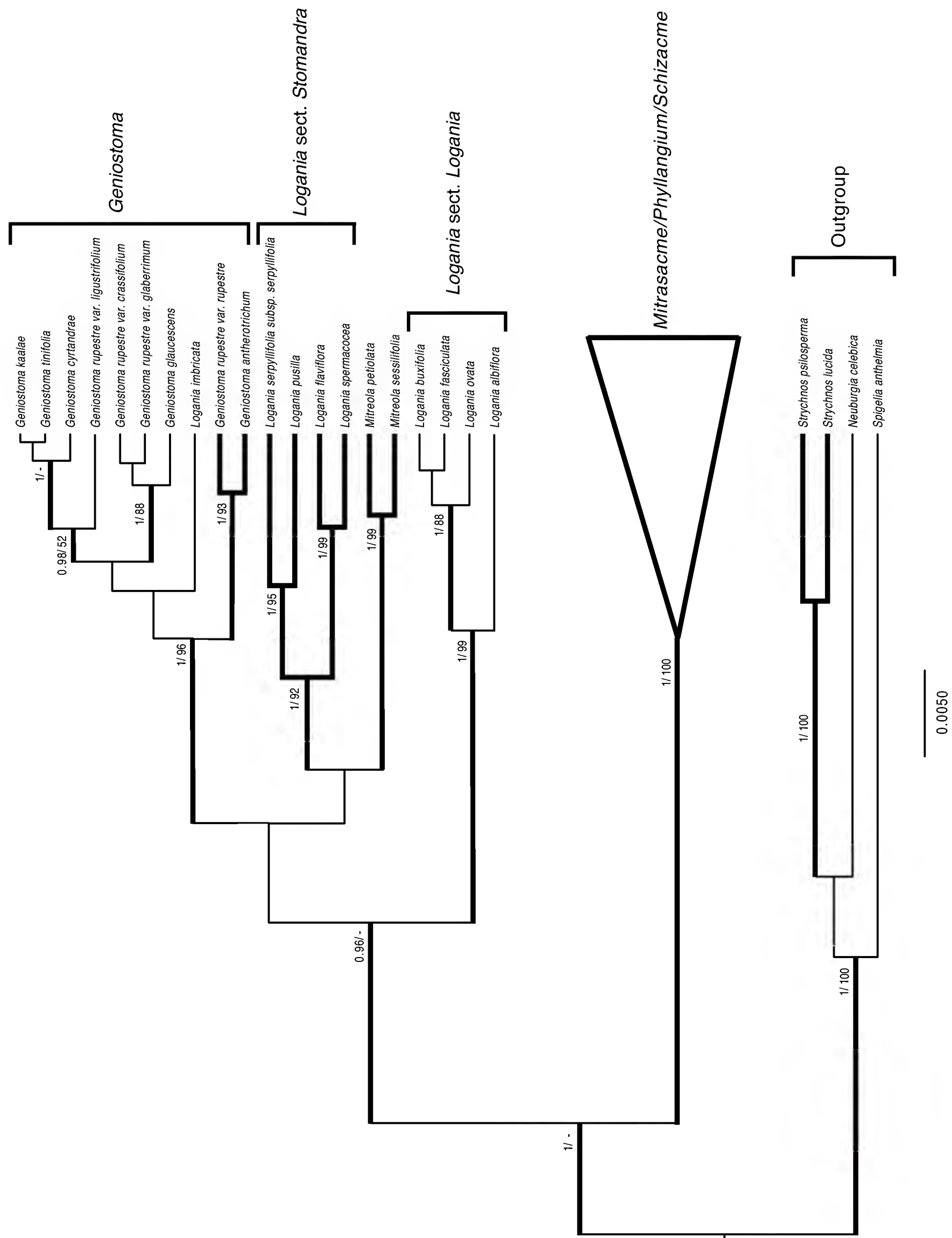


Fig. 1. Bayesian inference tree showing the phylogenetic relationships between *Logania imbricata* and other taxa within Loganiaceae. Posterior probabilities and bootstrap values are given above the branches (PP/BS); branches with support ≥ 0.95 PP are shown in bold; support values of $PP < 0.95$ and $BS < 70\%$ are not shown. Scale is in estimated substitutions per site. The data for this analysis were largely based on those of Gibbons et al. (2012).

3457, 20 Dec 1980 (MO, NSW244451); SE of summit of Mont Kouakoué, G.D. McPherson 19383, Y. Pillon, E.A. Brown & M. Gaudeul, 26 Apr 2006 (MO, NSW905713).

Affinities: the phylogeny of species of *Geniostoma* is still poorly known because insufficient taxa have been investigated. This species has a glabrous pistil and branchlets with enlarged nodes similar to those of *G. novae-caledoniae* Vieill. ex Baill., but lacks the undulate ‘wings’ on the internodes of that species and the tapering corolla buds are somewhat reminiscent of those of *G. balansaeorum* Baill. However, there is no evidence to suggest that *G. imbricatum* is likely to be closely related to either of these species.

Note: in the ‘Selected specimens examined’, the two names H.S. McKee and H.S. MacKee refer to the same plant collector. Hugh S. McKee changed the spelling of his family name to MacKee so that it would be easier for French-speakers to pronounce his name (K.L. Wilson, pers. comm., 30 Jan 2013).

Conclusions

Morphological and molecular data support the reassignment of *Logania imbricata* to *Geniostoma*, as *G. imbricatum*. Furthermore, the transfer of this taxon to *Geniostoma*, together with the above-mentioned extinction of the New Zealand *L. depressa*, results in the genus *Logania* being regarded as endemic to Australia.

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References

- Anonymous (2003+) ‘NZ Plant Conservation Network.’ (New Zealand Plant Conservation Network) http://www.nzpcn.org.nz/flora_search.aspx?scfSubmit=1&scfLatin_Name=logania+depressa (accessed 14 Jan 2013)
- Conn BJ (1980) A taxonomic revision of *Geniostoma* subg. *Geniostoma*. *Blumea* 26: 245–364.
- Conn BJ (1994) Revision of *Logania* R.Br. section *Stomandra* (R.Br.) DC. (Loganiaceae). *Telopea* 5: 657–692.
- Conn BJ (1995) Taxonomic revision of *Logania* section *Logania* (Loganiaceae). *Australian Systematic Botany* 8: 585–665. (<http://dx.doi.org/doi:10.1071/SB9950585>)
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214. (<http://dx.doi.org/doi:10.1186/1471-2148/7/214>)
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. (<http://dx.doi.org/10.1093/nar/gkh340>)
- Felsenstein J (1985) Confidence-limits on phylogenies — an approach using the bootstrap. *Evolution* 39: 783–791.
- Gibbons KL, Henwood MJ, Conn BJ (2012) Phylogenetic relationships in Loganieae (Loganiaceae) inferred from nuclear ribosomal and chloroplast DNA sequence data. *Australian Systematic Botany* 25: 331–340. (<http://dx.doi.org/10.1071/SB12002>)
- Guillaumin A (1953) Contributions à la Flore de la Nouvelle Calédonie. *Mémoires du Muséum National d’Histoire Naturelle, Paris, Série B, Botanique* 4: 44.
- Keane TM, Creevey CJ, Pentony MM, Naughton TJ, McInerney JO (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. *BMC Evolutionary Biology* 6: 29–48. (<http://dx.doi.org/10.1186/1471-2148-6-29>)
- Leenhouts PW, Steenis CGGJ, van (1962) Reduction of the genus *Nautophylla* Guillaumin to *Logania* R.Br. *Bulletin du Jardin botanique de l’État à Bruxelles* 32: 439–440.
- Lohne C, Borsch T (2005) Molecular evolution and phylogenetic utility of the petD group II intron: a case study in basal angiosperms *Molecular Biology and Evolution* 22: 317–332. (<http://dx.doi.org/10.1093/molbev/msi019>)
- Rambaut A, Drummond AJ (2009) ‘Tracer version 1.5.’ In <http://beast.bio.ed.ac.uk/Tracer> (accessed February 2011)
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739.